A Hemoglobin Based Oxygen Carrier, Bovine Polymerized Hemoglobin (HBOC-201) versus Hetastarch (HEX) in an Uncontrolled Liver Injury Hemorrhagic Shock Swine Model with Delayed Evacuation

Jennifer Gurney, MD, Nora Philbin, MSc, Jennifer Rice, MS, Françoise Arnaud, PhD, Feng Dong, MD, PhD, Meghan Wulster-Radcliffe, PhD, L. Bruce Pearce, PhD, Lewis Kaplan, MD, FACS, Richard McCarron, PhD, Daniel Freilich, MD

Background: **As HBOC-201 improves outcome in animals with hemorrhagic shock (HS), we compared HBOC-201 and HEX (used by U.S. military special operations forces) in a swine model of delayed evacuation and uncontrolled HS.**

Methods: **Twenty-four Yucatan pigs underwent a grade III liver injury and were resuscitated with HBOC-201, HEX, or no fluid (NON). Additional infusions were given for hypotension or tachycardia. After 4 hours, the liver was repaired; IV fluids and blood transfusions were administered. Pigs were monitored for 72 hours.**

Results: **Survival was 7/8, 1/8, and 1/8 in HBOC-201-, HEX-, and NON-resuscitated pigs, respectively. Compared with HEX, HBOC-201 pigs had higher systemic and pulmonary artery pressures and had comparable cardiac outputs, but were less tachycardic. Transcutaneous tissue oxygenation was restored more rapidly in HBOC-201 pigs, there was a trend to lower lactic acid, and base deficit was less. HBOC-201 pigs had lower fluid requirements, higher urine output, and lower blood loss than HEX pigs.**

Conclusions: **Despite evidence of vasoactivity, HBOC-201 more effectively stabilized tissue oxygenation, reversed anaerobic metabolism, decreased bleeding, and increased survival in comparison with HEX. If confirmed in clinical trials, these data suggest that for the resuscitation of combat casualties with delayed evacuation and uncontrolled HS due to solid organ injury, HBOC-201 is a superior low-volume resuscitative fluid.**

Key Words: **Combat casualty, Hemorrhagic shock, Swine, HBOC-201, Hextend, Liver injury, Uncontrolled hemorrhage.**

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Although disease and non-battle injuries are responsible for significant combat-related morbidity, trauma accounts for the preponderance of combat-related mortality. Recent casualties sustained in Operations Enduring and Iraqi Freedom demonstrate that trauma will continue to pose the most significant threat to U.S. military personnel in the 21st century. The most common causes of death that are potentially salvageable with optimal therapy are airway compromise, tension pneumothorax, and massive hemorrhage resulting in hemorrhagic shock (HS).[1] The current standard of care for the resuscitation of combat casualties with HS includes fluid infusion with asanguinous crystalloid or colloid solutions. However, while replenishing intravascular volume, these standard resuscitative fluids lack the ability to transport O2. Thus, even in urban trauma centers where pre-hospital transportation times are short, patients in HS arrive at the hospital with significant lactic acidosis and base deficit (BD) abnormalities, indicating on-going hypoperfusion.[2–4] As these abnormalities measured upon hospital arrival correlate with survival, multi-organ failure (MOF), and hospital length of stay, resuscitative strategies aimed at improving tissue oxygenation are likely to impact clinical outcome.[2,5–7]

In the unpredictable asymmetric battlefield of the War on Terrorism, transport times to definitive treatment, including surgery, critical care, and blood transfusion, are delayed.[8] Therefore, a "bridging" O2 carrying resuscitation fluid that can be infused during transport might ameliorate anaerobic metabolism and improve the physiologic status of casualties arriving at a higher echelon of care.[9]

The hemoglobin based oxygen carrier, HBOC-201 (Hemopure®, Biopure Corporation, Cambridge, MA), is an ultrapure, bovine-derived, polymerized Hb solution (Hb con-

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From the Walter Reed Army Medical Center (J.G.), Washington, DC; the Naval Medical Research Center (N.P., J.R., F.A., F.D., R.M., D.F.), Silver Spring, Maryland; Purdue University (M.W.-R.), West Lafayette, Indiana; Biopure Corp. (L.B.P.), Cambridge, Massachusetts; and Yale University School of Medicine (L.K.), New Haven, Connecticut.

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Address for reprints: Nora Philbin, MSc, 2N77 503 Robert Grant Avenue, Combat Casualty Care, Naval Medical Research Center, Silver Spring, MD 20910; email: philbinn@NMRC.NAVY.MIL

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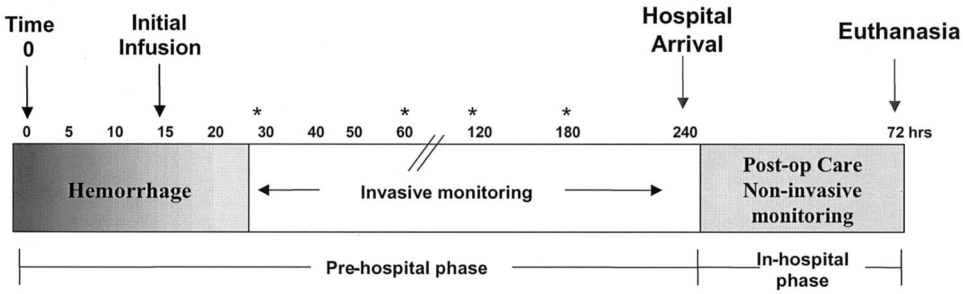

Fig. 1. *Experimental design. Liver injury denoted start of the experiment (Time 0). Fluid resuscitation was initiated at 15 minutes, and additional infusions (*) were provided for MAP < 60 mmHg or HR > baseline. Pre-hospital care was simulated between 15 and 240 minutes, and hospital arrival was simulated at 240 minutes, at which time surgical sites were repaired, PRBCs and/or NS infused, and animals recovered from anesthesia. Animals were euthanized at 72 hours.*

centration of 13 g/dL) with a pH of 7.82 (range 7.6–7.9) and an osmotic pressure similar to whole blood. HBOC-201 is universally compatible, stable at 2–40°C for ≥18 and 2–30°C for ≥3 months, respectively, and can be administered easily by simple intravenous administration without special training or medical expertise.

The aim of the study reported herein was to confirm our hypotheses that in a swine model simulating combat casualties with uncontrolled hemorrhage due to a solid organ injury incorporating delayed arrival to definitive care, HBOC-201 would stabilize hemodynamics, increase tissue oxygenation, diminish lactic acidosis, and decrease morbidity and mortality in comparison with hetastarch.

MATERIALS AND METHODS

The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animals Resources, National Research Council, National Academy Press, 1996. The study was approved by the WRAIR/NMRC Institutional Animal Care and Use Committee (IACUC) and all procedures were performed in an animal facility approved by the Association for Assessment and Accreditation for Laboratory Animal Care International (AAALAC).

Animal Preparation

Twenty-four male and female Yucatan Mini pigs (~25 kg) (Sinclair Research Center, Inc., Columbia, MO) were used. Feed and water were withheld 12–14 hours before initiation of the experiment. Animals were sedated and anesthesia induced with intramuscular ketamine hydrochloride (33 mg/kg), atropine sulfate (0.05 mg/kg), and mask ventilation with isoflurane (3.0%) and 100% O_2 to facilitate endotracheal intubation. Anesthesia was maintained via isoflurane (1%-2.5%) in 21% O_2. Pigs were ventilated for anesthesia-induced apnea (Ohmeda 7800 series ventilator, Datex, Madison, WI) (12–15 breaths/min; tidal volume 5–10 mL/kg; and FiO_2 0.21). Data from animals unable to regain spontaneous breathing before resuscitative fluid administration were excluded (n = 2). Body temperature (BT) was monitored and supported. Urine was collected via bladder catheterization. The right external jugular vein and carotid artery were dissected and isolated. An 8 F introducer sheath was placed in the external jugular vein using Seldinger technique and a 7.5 F pulmonary artery catheter (PAC; Edwards Life Sciences, Irvine, CA,) was inserted for continuous hemodynamic and cardiac output (CO) monitoring. A 20G Angiocath was placed in the carotid artery and mean arterial pressure (MAP) was continuously transduced. A midline laparotomy was performed to expose the liver and define the four major lobes. The abdominal viscera were packed and a malleable retractor was used to identify and isolate the lower left lobe. All surgical procedures were performed under aseptic techniques.

Injury, Hemorrhage, and Resuscitation Procedures

A standardized liver injury was created by placing a ring clamp over the left lower lobe, ~50% in width and ~0.75–2.0" from the apex, adjusting for relative size of the liver and weight of the pig. The clamp was closed and an 11 blade was used to lacerate the lobe from the top of the clamp through the remaining width. The liver injury denoted the start of the pre-hospital phase (Time 0). After 1 minute, the clamp was removed and the remaining tissue excised, resulting in ~25% lobectomy, consistent with a grade III liver injury.[10] Bleeding was spontaneous, unhampered, removed via intraperitoneal suction, and quantified by weight.

Pigs were randomly allocated to one of three treatment groups: HBOC-201; 6% hetastarch in LR (HEX, Hextend®, Abbott Laboratories, Abbot Park, IL); or no fluids (NON) (Fig. 1). At 15 minutes, resuscitated pigs were administered 10 mL/kg of HBOC-201 or HEX over 10 minutes. Additional infusions of 5 mL/kg were provided at 30, 60, 120, and 180 minutes post-injury if hypotension (MAP < 60 mm Hg) or tachycardia (HR > baseline value [Time 0]) were observed. Fluids were infused at room temperature. At 60 minutes, blood collection was discontinued and the abdomen was closed with towel clips.

Table 1 Demographics and Baseline Data (Means ± SEM)

Group	Weight, kg	Sex Ratio, M/F	Hemorrhage Volume, mL	EBV, %
HBOC-201	22.7 ± 2.7	4:4	369.5 ± 41.5	26.0 ± 2.1
HEX	23.6 ± 10.4	4:4	498.3 ± 43.1	38.6 ± 6.6
NON	23.6 ± 8.5	2:6	496.1 ± 46.4	34.6 ± 4.0

No significant differences were observed.

Recovery and In-Hospital Phase

Hospital arrival was simulated at 4 hours. The abdomen was reopened, residual blood was suctioned, sponges collected, and blood loss quantified by weight; 10 mL/kg allogeneic packed red blood cells (PRBC; for Hb < 7 g/dL), 20 mL/kg normal saline (NS), 13 mg/kg cephazolin (antibiotic), and 0.01 mg/kg buprenorphine (analgesic), were administered. The PAC was removed, jugular vein introducer, secured for postoperative blood sampling and fluid administration, and arterial and bladder catheters removed. Surgical incisions were closed and surgical dressings applied. Animals were extubated and recovered from anesthesia.

Long-Term Survival Procedures

Vital signs and general status were assessed 24, 48, and 72 hours post-injury. Pigs received 10 mL/kg NS, 10 mL/kg PRBCs as needed for anemia, antibiotics, and analgesia. Pigs were euthanized 72 hours post-injury for necropsy and histologic analysis.

Data Collection

Standard invasive and noninvasive hemodynamic parameters were monitored for 240 minutes during the simulated prehospital phase (Fig. 1). Blood loss was measured by weighing collection canisters at 5 and 15 minutes (pre-resuscitation), and 20, 30, 60, and 240 minutes (post-resuscitation). Sponge weight was included in total post-resuscitation blood loss. Transcutaneous tissue oxygenation (TCOM or $tcpO_2$) was noninvasively measured with a TCM4 Tina monitor (Radiometer, Copenhagen, Denmark) using four Clark type polarographic electrodes (data represent mean values) positioned bilaterally on the upper torso and on the inner thighs. Blood gases (ABG and MVBG) were measured with an automatic analyzer (ABL 705, Radiometer, Copenhagen, Denmark). Blood samples were collected for complete blood counts (CBC, Pentra 60 C+, ABX, France), chemistries (Vitros 250 Analyzer, Ortho).

Statistical Analysis

Statistical analysis was performed using student's *t* test for data between groups at specific time points and the GLM procedures of SAS (SAS Inst., Inc., Cary, NC) were used to analyze data collected over time. The GLM model included terms treatment, pig nested within treatment time and treatment x time. Pig nested within treatment was the main plot error term, and the residual was the subplot error term. The variance associated with the main plot error term was used to calculate overall SEM associated with main plot variables. When appropriate, the PDIFF (i.e. a method for comparing all possible least squares means) option in SAS was used to compare individual means. Data are expressed as mean ± SEM (SEM) for animals alive at time of measurement.

RESULTS

Baseline body weight, sex distribution, and initial hemorrhage volume (mL and % estimated blood volume [EBV]) (Table 1), as well as hemodynamics, metabolic parameters, and tissue oxygenation were not different between groups.

Hemorrhagic Shock

Liver injury with uncontrolled hemorrhage resulted in a 60.3% decrease in MAP (69.6 ± 3.2 to 27.6 ± 2.9 mm Hg), 55.4% decrease in cardiac index (CI) (5.6 ± 0.5 to 2.5 ± 0.3 mL/beat/min^2), 77.7% decrease in tcpO2 (14.8 ± 2.2 to 3.3 ± 1.2 mm Hg), and a 22.8% increase in HR (141.3 ± 4.7 to 173.5 ± 7.8 bpm). Combined mean blood loss in the first 15 minutes was 21.4 ± 1.8 mL/kg or EBV 32.6 ± 2.8%.

Hemodynamics

Resuscitation with HBOC-201 stabilized hemodynamic parameters (Fig. 2). In contrast to HEX-resuscitated pigs, MAP in HBOC-201 pigs stabilized more rapidly and there was a significant effect of treatment over time ($p < 0.001$). MAP was restored to baseline in HBOC-201 animals at 120 minutes but failed to return to baseline in surviving HEX or NON animals during the pre-hospital phase. HBOC-201 pigs were less tachycardic during the pre-hospital phase. At 45 minutes, HR was 176.8 ± 9.3 bpm in HBOC-201 and 209.7 ± 0.1 bpm in HEX pigs ($p = 0.04$) and 228.0 ± 16.3 bpm in NON pigs ($p = 0.01$). MPAP and SVRI were higher in HBOC-201 than HEX pigs throughout the pre-hospital phase. At 45 minutes, MPAP was 17.5 ± 4.7 versus 7.5 ± 3.9 mm Hg and SVRI was 1854.5 ± 118.1 versus 1133.2 ± 148.5 dynes*sec*m2/cm5 ($p < 0.001$) in HBOC and HEX, respectively. CI was similar in HBOC-201 and HEX pigs and returned to baseline by 150 minutes. An effect of treatment over time was observed for oxygen delivery (DO2) ($p = 0.006$) such that HBOC-201 pigs had consistently higher DO2 (Table 2) but, no effects on oxygen consumption (VO2) or the oxygenation extraction ratio (O2ER) were observed (Table 2).

Blood Loss Index

Post-resuscitation blood loss up to 60 minutes was 18.9 ± 4.5, 27.3 ± 5.2, and 17.0 ± 1.9 mL/kg/survival hour in HBOC-

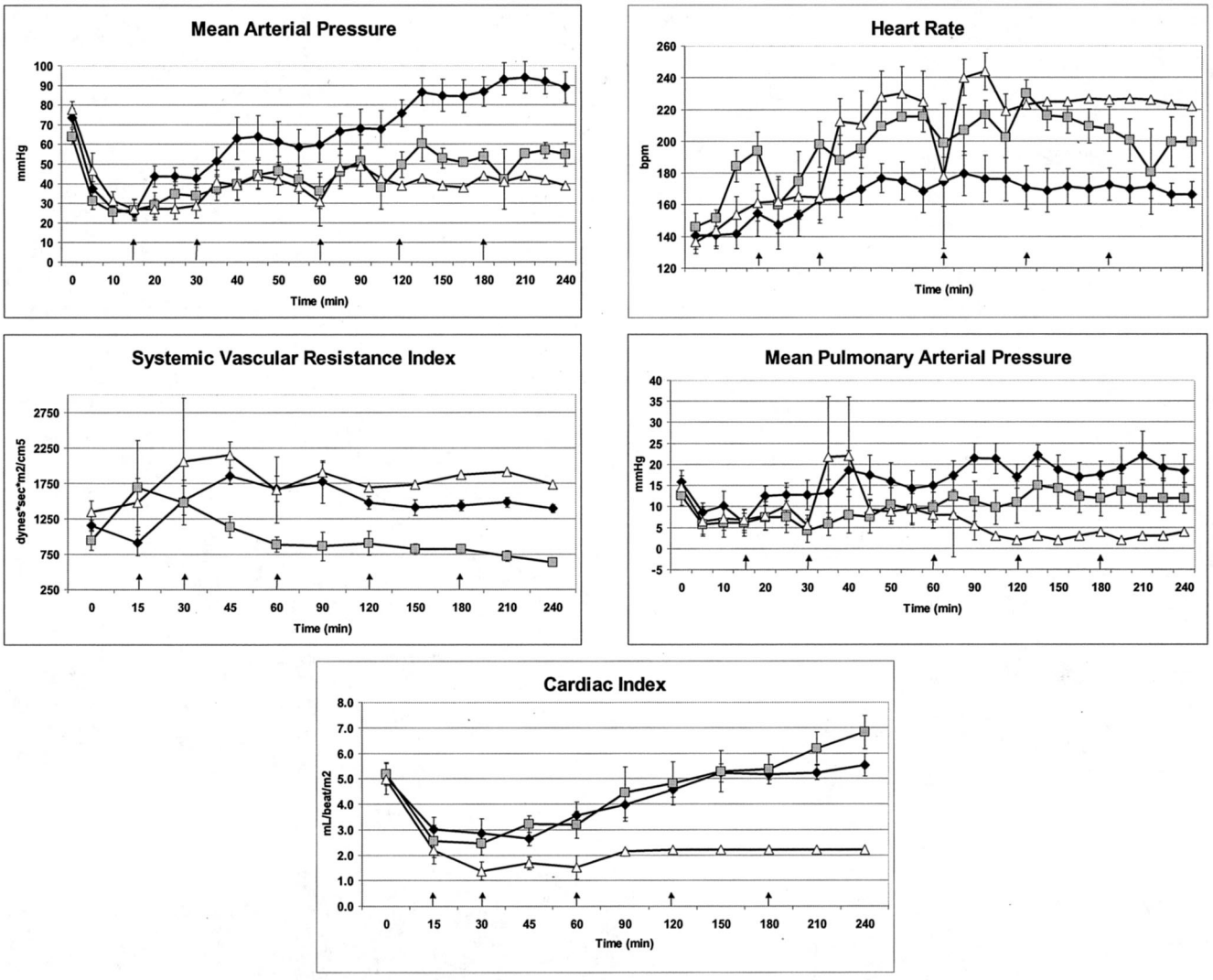

Fig. 2. *Hemodynamic changes in swine with uncontrolled hemorrhage resuscitated with HBOC-201 (Hemopure®), HEX (6% Hetastarch in balanced salt solution, Hextend®), or NON (no resuscitation). Arrows indicate times of infusion. HBOC-201 (—◆—), HEX (—▦—), and NON (—△—).*

201, HEX, and NON groups, respectively (p = NS) (Fig. 3). Respective blood loss during the pre-hospital phase (resuscitation to 240 minutes) was 6.9 ± 2.3, 21.8 ± 6.4, 14.6 ± 2.5 mL/kg/survival hour (HBOC-201 versus HEX, $p = 0.04$). Total blood loss was lower in HBOC-201 (8.2 ± 12.3 mL/kg/survival hour) as compared with HEX (24.3 ± 7.0 mL/kg/survival hour)

Table 2 Oxygen Status

Variable (mean ± SEM)	Resus Group	T 0	T 30	T 60	T 180	T 240
DO_2^	HBOC-201	621.1 ± 39.6	341.1 ± 72.0	416.5 ± 99.2	597.2 ± 46.9	710.2 ± 117.1
	HEX	677.6 ± 63.8	244.8 ± 48.6	297.0 ± 80.2	356.3 ± 62.9	408.3 ± 12.7
	NON	700.9 ± 106.2	198.3 ± 65.1	299.7 ± 86.8	370.6 ± 0.0	373.5 ± 0.0
VO_2	HBOC-201	103.3 ± 16.2	152.2 ± 37.5	144.2 ± 17.8	140.9 ± 7.4	182.5 ± 23.3
	HEX	123.1 ± 14.4	136.8 ± 42.7	134.4 ± 34.3	125.8 ± 40.0	93.0 ± 37.3
	NON	119.7 ± 18.8	123.8 ± 50.9	130.0 ± 43.3	178.9 ± 0.0	193.6 ± 0.0
O_2ER	HBOC-201	16.4 ± 2.0	46.1 ± 4.4	52.0 ± 15.6	24.5 ± 2.2	27.5 ± 3.8
	HEX	18.1 ± 1.3	56.1 ± 8.7	54.9 ± 7.7	33.2 ± 6.0	22.9 ± 9.1
	NON	17.1 ± 1.6	60.8 ± 11.7	59.1 ± 5.3	48.3 ± 0.0	51.8 ± 0.0

^ Overall $p < 0.05$.

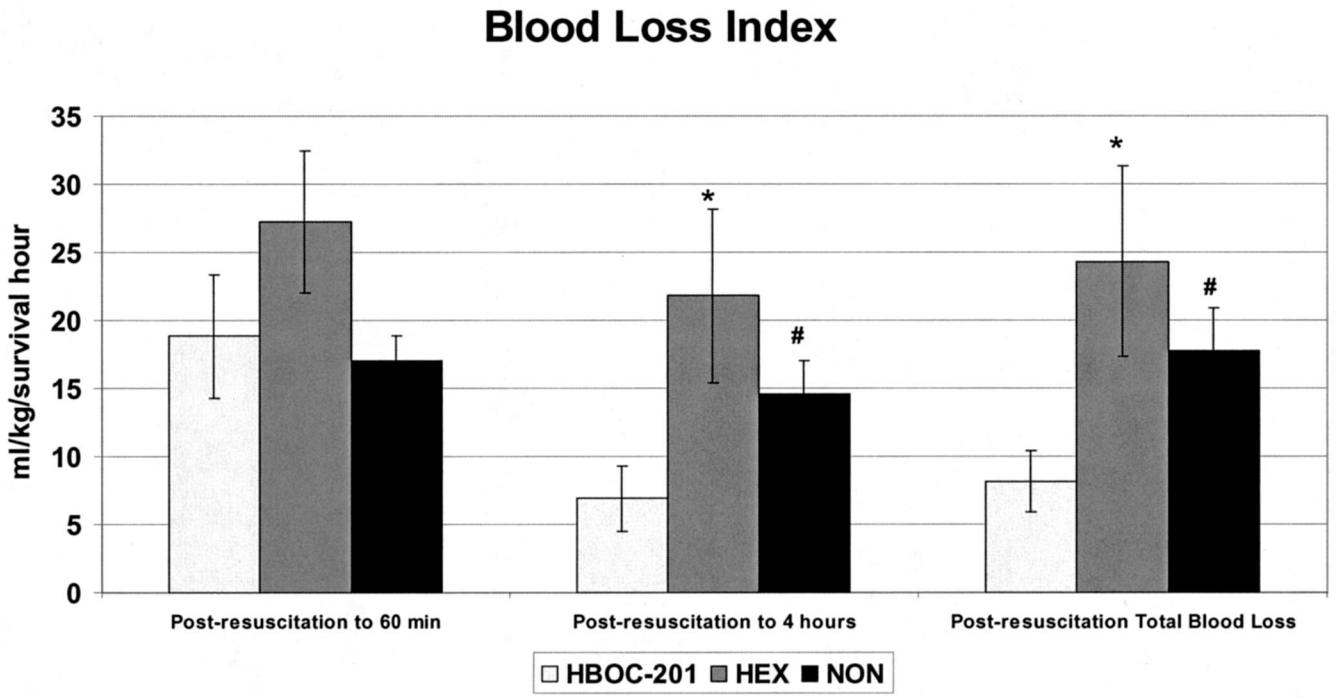

Fig. 3. *Blood loss index (mL/kg/survival hour) over the 4-hour pre-hospital phase. Post-resuscitation blood loss up to 60 minutes was comparable (left). Post-resuscitation blood loss to 4 hours (center) and total post-resuscitation blood loss (right) was lower in HBOC-201 than HEX or NON pigs. *HBOC-201 vs. HEX,* $p < 0.05$*; #HBOC-201 vs. NON,* $p < 0.05$ *by Student's* t *test.*

($p = 0.04$) or NON pigs (17.7 ± 3.2 mL/kg/survival hour) ($p = 0.03$). The largest difference in blood loss between HBOC-201- and HEX-resuscitated pigs was observed at 60 minutes (7.44 ± 8.7 versus 11.3 ± 9.8 mL/kg, respectively). Nadir total hemoglobin levels were 9.22 ± 0.31 g/dL (180 minutes), 4.43 ± 0.38 g/dL (240 minutes), and 9.78 + 0.68 g/dL (30 minutes) in the HBOC, HEX, and NON groups, respectively (Table 3).

Fluid and Blood Transfusion Requirements

Fluid requirements were lower in HBOC-201 than HEX pigs at 60, 120, and 180 minutes (Table 4). Total fluid index was 7.0 ± 0.8 versus 15.5 ± 2.8 mL/kg/survival hour in the HBOC-201 and HEX groups, respectively ($p = 0.01$) (Fig. 4). At the simulated hospital arrival (240 minutes), 0/7 and 3/3 surviving HBOC-201 and HEX pigs, respectively, met criteria for and received PRBC transfusions.

Renal Function

HS resulted in oliguria and/or anuria in all groups. Urine output resumed by 90 minutes in HBOC-201 pigs as compared with 210 and 240 minutes in HEX and NON pigs, respectively. Total pre-hospital urine output was similar in HBOC-201, HEX, and NON pigs (1.1 ± 0.7, 0.6 ± 0.4, and 0.1 ± 0.1 mL/kg/h, respectively) (p = NS) (Fig. 5). Creatinine levels were similar in the HBOC-201 and HEX groups at 48 and 72 hours (Table 5). BUN was lower after 180 minutes in HBOC-201 pigs compared with HEX pigs. Hyperkalemia occurred only in NON pigs. At 30 and 60 minutes, potassium was lower in HBOC-201 compared with NON pigs ($p = 0.04$ and $p = 0.004$, respectively).

Indirect and Direct Measures of Tissue Oxygenation

Lactic acid at 60 minutes was 3.2 ± 0.7, 6.7 ± 4.4, and 11.0 ± 1.7 mmol/L in HBOC-201, HEX, and NON pigs, respectively (HBOC-201 versus HEX [$p = 0.08$], HBOC-201 versus NON [$p = 0.001$]) (Fig. 6). There was a trend toward more rapid lactate clearance in HBOC-201 pigs but early mortality in HEX and NON pigs precluded definitive analysis. Base excess (inverse of BD) was higher in HBOC-201 than HEX and NON pigs ($p = 0.004$). At 90 minutes, BE was 2.1 ± 2.6, -4.8 ± 2.8, and -8.0 ± 0.1, respectively (Table 6). pH and bicarbonate were similar in all groups. Mixed venous

Table 3 **Total Hemoglobin, g/dL (Means ± SEM)**

Resus Group	T 0	T 30	T 60	T 180	T 240
HBOC-201	9.67 ± 0.38	9.38 ± 0.55	9.89 ± 0.52	9.22 ± 0.31	10.07 ± 0.66
HEX	10.11 ± 0.17	7.55 ± 0.36	7.86 ± 0.61	5.00 ± 0.57	4.43 ± 0.38
NON	10.32 ± 0.33	9.78 ± 0.68	11.32 ± 0.78	12.70 ± 0.0	12.80 ± 0.0

Table 4 Fluid Requirements

Time point	# of Animals Requiring Infusion/# of Animals Alive	
	HBOC-201	HEX
15 min	8/8 (100%)	8/8 (100%)
30 min	8/8 (100%)	7/7 (100%)
60 min	6/8 (75%)	5/5 (100%)
120 min	5/7 (71%)	3/3 (100%)
180 min	4/7 (57%)	3/3 (100%)

p = NS.

O2 (SVO2) failed to return to baseline in any group, but was higher in HBOC-201 than HEX pigs at 60 minutes ($p = 0.05$) (Table 6). Partial pressure of O2 (po2) was higher in HBOC-201 as compared with HEX pigs at 60 minutes ($p = 0.03$).

HBOC-201 resuscitation resulted in higher tcpO2 than HEX or NON ($p = 0.02$). At 135 minutes, tcpO2 was 29.5 ± 7.4 mm Hg in HBOC-201 and 13.8 ± 8.0 mm Hg in HEX pigs ($p = 0.02$) (Fig. 7).

Survival

Survival to simulated hospital arrival (240 minutes) was 7/8 (87.5%), 3/8 (37.5%) and 1/8 (12.5%) in HBOC-201-, HEX- and NON-resuscitated pigs (HBOC-201 versus NON $p = 0.01$). Respective survival rates to 72 hours were 7/8 (87.5%), 1/8 (12.5%) and 1/8 (12.5%) (HBOC-201 versus HEX $p = 0.01$) (Fig. 8).

DISCUSSION

In our model of uncontrolled hemorrhage due to a solid organ injury, HBOC-201 clearly was a superior hypotensive resuscitative fluid than 6% hetastarch (Hextend, HEX). HBOC-201 stabilized hemodynamics, restored tissue oxygenation, diminished base deficit, maintained urine output, and markedly decreased mortality. Moreover, despite evidence of mild vasoactivity, we have shown for the first time that low volume resuscitation with HBOC-201 does not increase bleeding. Thus, at least for solid organ injury induced HS, it appears that HBOC-201 is superior to HEX, the current resuscitative fluid used by U.S. special operations forces, and clinical trials to corroborate these findings in humans are warranted.

In 2003, the optimal resuscitation strategy for the trauma patient in HS is unclear, but appears to depend on the patient subgroup. For patient with HS in whom hemorrhage has been controlled, aggressive fluid resuscitation clearly stabilizes hemodynamics, increases tissue perfusion, and improves clinical outcome. However, recent data question the benefits of fluid resuscitation when hemorrhage has not been controlled. The theoretical concerns are that fluid resuscitation in uncontrolled hemorrhage will increase bleeding due to thrombus dislodgement secondary to increased arterial pressure. This event would be complicated by hemodilution of platelet and coagulation factors thereby increasing thrombocytopenia and coagulopathy. In fact, these hypothetical concerns have been confirmed in preclinical studies and somewhat supported by data generated in clinical trials. In numerous animal models of uncontrolled hemorrhage, aggressive fluid resuscitation with asanguinous crystalloid or colloid solutions has been shown to increase hemorrhage and reduce survival.[11–15]

In the landmark Houston clinical trial, a survival benefit was demonstrated with delayed hypotensive resuscitation in comparison with immediate hypotensive resuscitation (70% versus 62%, $p = 0.04$).[3] However, the survival benefit was small and conclusions may not be generalizable to the broader trauma population. Enrollment was restricted to penetrating trauma patients, 8% of delayed resuscitation patients were aggressively resuscitated before surgical hemorrhage control (study deviations), and fluid resuscitation was not titrated to clinical response (standard of care). Potentially

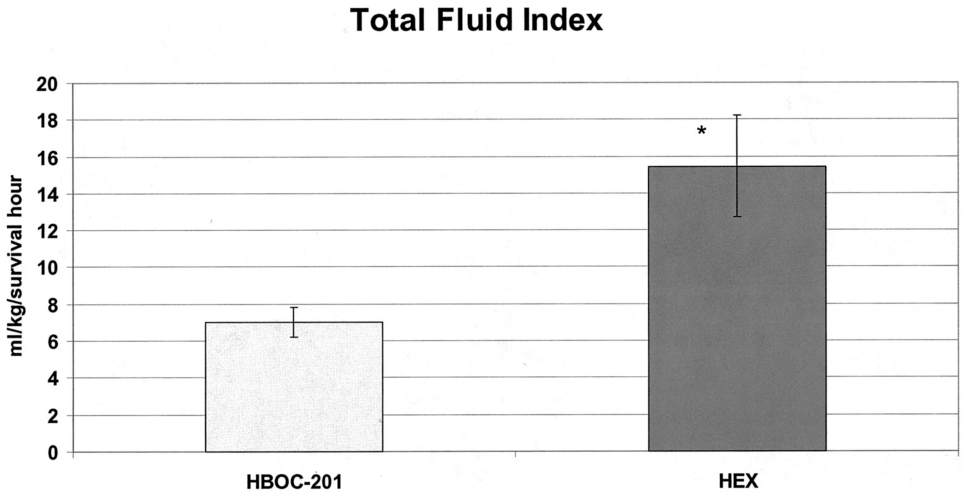

Fig. 4. *Total pre-hospital fluid requirement index (mL/kg/survival hour). HBOC-201 pigs required less fluid compared with HEX pigs. *HBOC-201 vs. HEX,* p = *0.01 by Student's* t *test.*

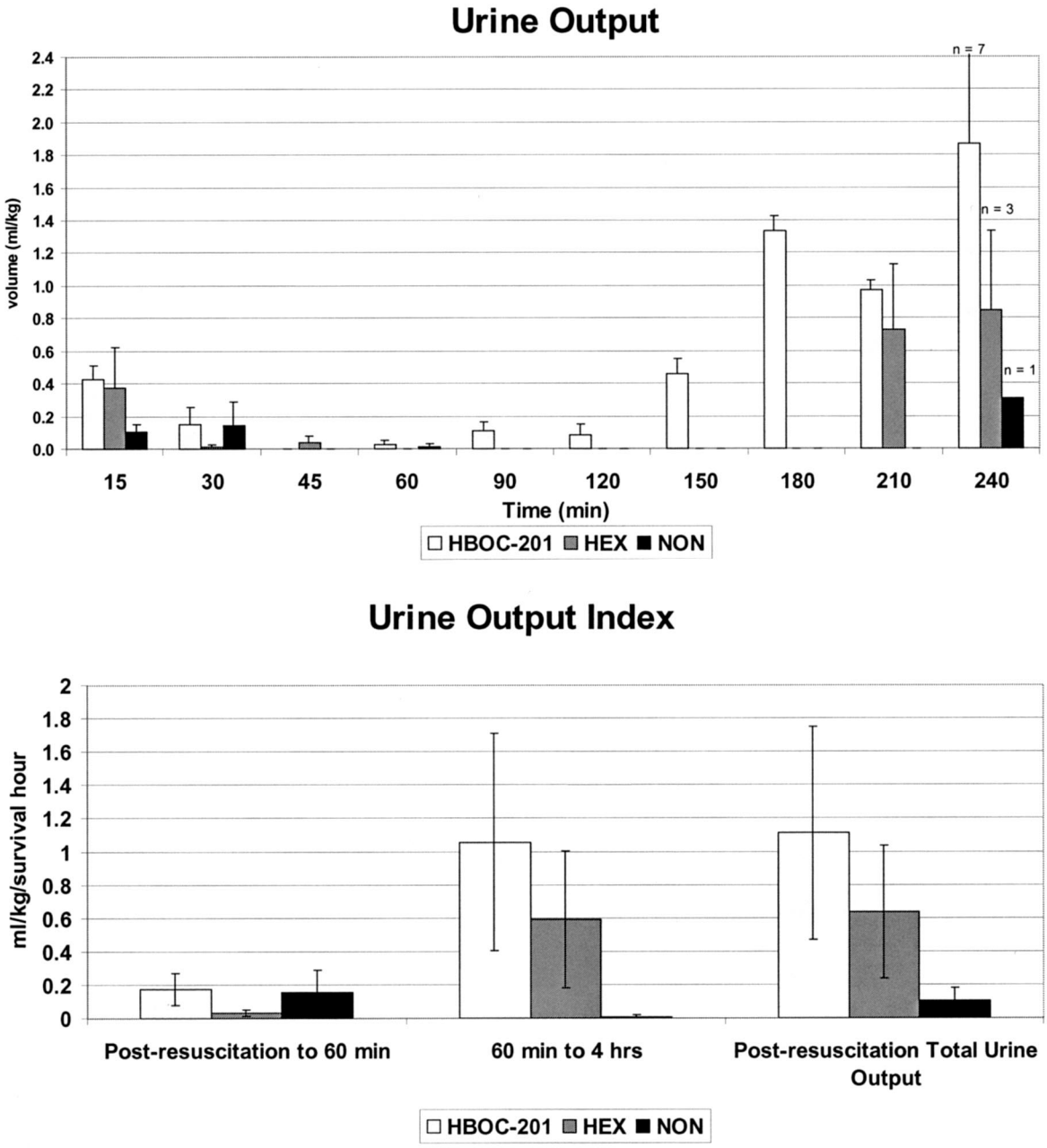

Fig. 5. *Pre-hospital urine output (mL/kg) (top) and urine output index (mL/kg/survival hour). HBOC-201 pigs had resumption of urine output (mL/kg) by 90 minutes as compared with 210 and 240 minutes in the HEX and NON groups, respectively (top). Urine output index was comparable in all three groups*

suboptimal study design included an alternate day allocation method and absence of blinding during outcome analysis.

In the Baltimore trial, comparing normotensive and hypotensive resuscitation strategies, clinical outcome was similar in both groups. Although the investigators intended to compare resuscitation strategies targeting systolic blood pressures (SBP) of 100 and 70 mm Hg, respectively, spontaneous resolution of hypotension occurred in this population of predominantly surviving patients. Respective SBPs during active hemorrhage were actually 114 and 100 mm Hg, thus diminishing the power of the study to detect a difference in outcome between the two groups.[16] Importantly, a recent Cochrane review concluded that currently there is "no evidence from randomized controlled trials for or against early or larger volume of intravenous fluid administration in uncontrolled hemorrhage."[17]

Based on recently published studies, the potential benefits of hypotensive/low volume resuscitation reported with standard fluids can be extrapolated to HBOCs. It appears that hypotensive/low volume resuscitation with HBOCs may im-

Table 5 Renal Function

Variable, (mean ± SEM)	Resus Group	T 0	T 30	T 60	T 180	T 240	T 48H	T 72H
Creatinine, mg/dL	HBOC-201	0.8 ± 0.1	NA	NA	NA	NA	0.7 ± 0.1	0.6 ± 0.1
	HEX	0.8 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.3 ± 0.2	1.3 ± 0.2	0.8 ± 0.0	0.8 ± 0.0
	NON	0.8 ± 0.1	1.0 ± 0.1	1.3 ± 0.1	ND	ND	0.6 ± 0.0	0.6 ± 0.0
BUN, mg/dL	HBOC-201	12.8 ± 1.9	15.1 ± 1.6	16.4 ± 1.8	18.3 ± 1.4*	19.2 ± 2.0		
	HEX	15.4 ± 1.4	16.7 ± 1.3	17.1 ± 1.5	24.7 ± 2.0	26.3 ± 2.0		
	NON	13.4 ± 1.2	15.5 ± 1.4	14.3 ± 0.3				
Potassium, mmol/L	HBOC-201	3.9 ± 1.0	4.8 ± 0.2#	4.9 ± 0.4#	4.8 ± 0.2			
	HEX	5.0 ± 0.1	5.4 ± 0.3	5.1 ± 0.5	4.8 ± 0.4			
	NON	5.4 ± 0.9	6.1 ± 0.6	8.3 ± 1.0				

* HBOC vs. HEX, $p < 0.05$.
\# HBOC vs. NON, $p < 0.05$.
ND, no data; NA, interference by HBOC-201.

prove outcome in comparison with non-O_2 carrying resuscitative fluids. The Carolina Resuscitation Research Group compared hypotensive resuscitation with HBOC-201 and LR in a simulated uncontrolled hemorrhage liver injury swine model, targeting a MAP of 60 mm Hg.[18] Blood pressure was higher, lactic acidosis was lower, and short-term survival was improved with HBOC-201. The investigators extended their findings in a second study comparing hypotensive resuscitation with HBOC-201 and 6% hetastarch, in which hemodynamics were stabilized and survival was dramatically improved in the HBOC-201 group.[19] The volume and rate of post-resuscitation hemorrhage was not reported.

U.S. Air Force investigators demonstrated that hypotensive resuscitation with HBOC-201 targeting a MAP of 60 mm Hg reduced lactic acidosis in comparison with normotensive resuscitation with LR.[20] Importantly, reversal of anaerobic metabolism was not observed with HBOC-201 with a target MAP of 50 mm Hg. It appears that 60 mm Hg may be the minimum pressure at which tissue perfusion is maintained to an extent demonstrable by measurable reductions in LA. In a second study in a similar controlled HS model, the same investigators extended their findings to other resuscitation fluids, showing that low volume resuscitation with HBOC-201 reversed anaerobic metabolism at significantly lower volumes than hypertonic saline 7.5% with or without Dextran-70 (HTS 7.5% and HSD), pentastarch 6%, and hetastarch 6%.[21]

We sought to evaluate HBOC-201 in a clinically relevant and militarily realistic swine model of uncontrolled hemorrhage. We used the liver crush/laceration model of Manning et al.,[18] adapting it to better simulate the austere pre-hospital environment of medical care typical in combat, including: (a) spontaneous ventilation at an FiO_2 of 0.21 instead of 1.0 because O_2 is not universally available; (b) unrestricted hemorrhage from the liver injury; (c) quantification of blood loss; (d) simulation of limited fluid availability versus continuous titrated fluid infusions; and (e) prolongation of simulated pre-hospital time to four hours. The model is reproducible, severe (mortality 87.5% in NON-resuscitated animals), and useful for the evaluation of resuscitation fluids for the military.

We found that key surrogates of morbidity and mortality were improved in HBOC-201-resuscitated pigs. Although blood pressure is an insensitive indicator of the severity of shock, admission blood pressure correlates with survival.[22] Consistent with previous reports, MAP was rapidly restored in HBOC-201 pigs, presumably related to HBOC-201's volume expanding and vasoactive properties—especially nitric oxide binding.[23,24] Despite mild systemic and pulmonary hypertension and relative bradycardia, in contrast to the findings of Sampson et al. in a controlled HS model, cardiac output was comparable in HBOC-201- and HEX-resuscitated pigs.[21] That increased blood pressure was observed without diminished cardiac output or increased bleeding, supports the clinical utility of HBOC-201 resuscitation in severe HS.

It is apparent that as an O_2 carrier in severe HS, HBOC-201 transports O_2 to peripheral tissues and reduces anaerobic metabolism. As shunting of blood from the integument is an early manifestation of physiologic compensation to HS, cutaneous oxygenation should be a sensitive indicator of global perfusion. In comparison with HEX, HBOC-201 resuscitation resulted in higher and more rapid recovery of tissue oxygenation, a trend to lower lactate levels and more rapid lactate clearance, and significantly lower base deficit. As these parameters correlate with morbidity (ARDS, MOF) and mortality in animal and human studies,[25–27] the improvements observed with HBOC-201 in swine can be expected to translate into a clinical benefit in humans.

O_2 delivery has been reported to be relatively lower with HBOC-201 resuscitation.[21] On the positive side, HBOC-201 increases blood O_2 content, and HBOC-201's elevated P50 (38 vice 26.5 mm Hg for blood) increases O_2 release at the tissue level and can increase O_2 extraction.[28] On the negative side, HBOC-201 increases systemic and pulmonary vascular resistance (increased after load), can decrease cardiac output, and thus, can have a net negative effect on O_2 delivery.[21]

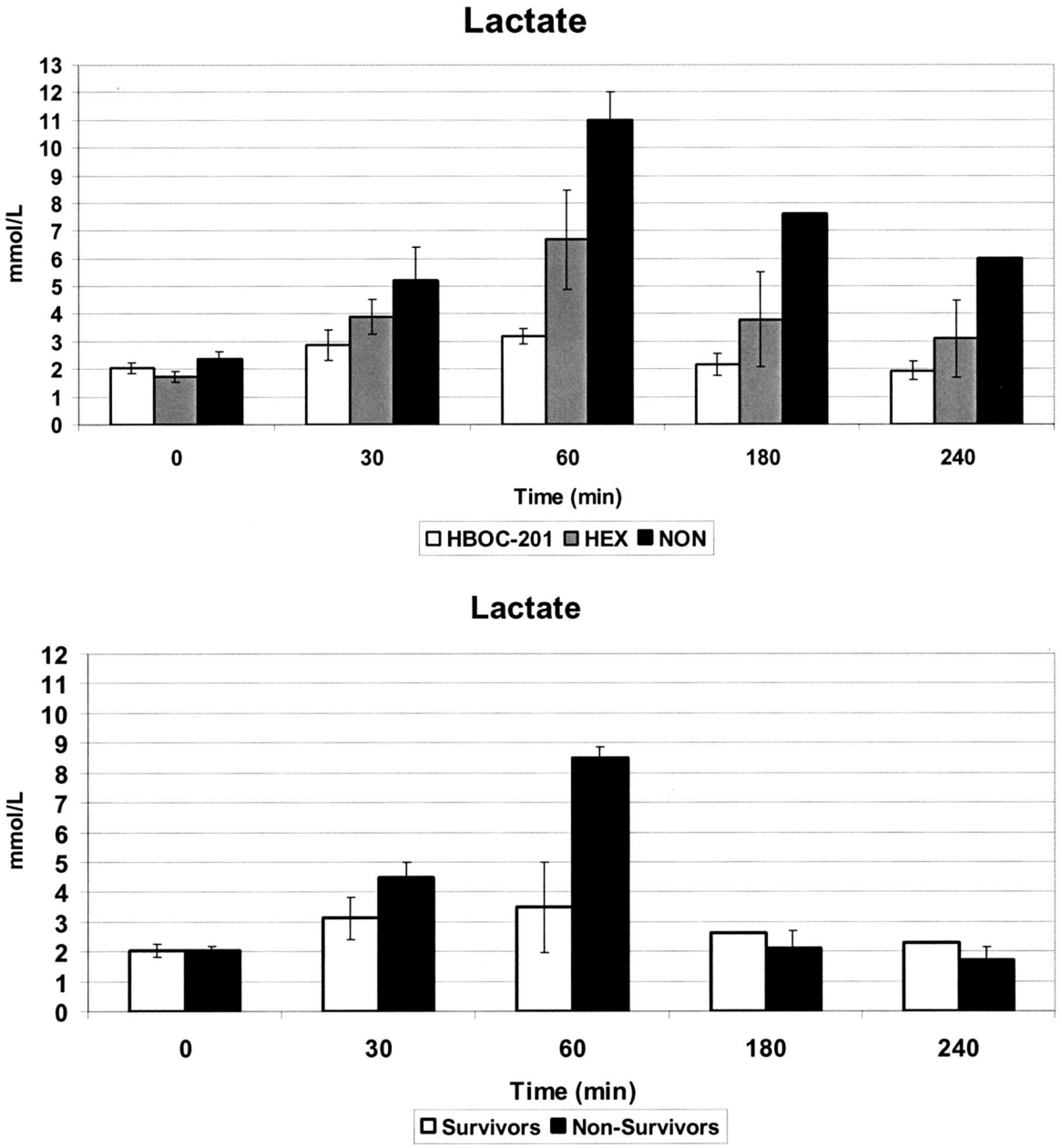

Fig. 6. *Blood lactic acid. At 60 minutes, lactic acid was lowest in HBOC-201–resuscitated pigs (top) (HBOC-201 vs. HEX,* $p = 0.08$*; HBOC-201 vs. NON,* $p < 0.05$*). Lactic acid in survivors vs. non-survivors is also shown (bottom).*

However, in our severe uncontrolled HS model, cardiac output was not diminished and O_2 delivery was higher in HBOC-201-resuscitated pigs. In addition to O_2 delivery, optimizing O_2 consumption and cardiac output have been suggested as goals for resuscitation,[29–31] but these parameters were comparable in our study, suggesting that other indicators of hypoperfusion will be important for the clinical care of patients receiving HBOC-201.

Our cutaneous tissue oxygenation data support reports by other investigators which showed improved brain tissue oxygenation in HS upon resuscitation with HBOC-201 and ventilation with 100% O_2.[32,33] At first glance, our data contradict Knudson et al. 's report that showed equivalent deltoid and liver O_2 levels with HBOC-201, LR, and HSD, in HS; however, swine were mechanically ventilated with 100% O_2 in that study[34] versus spontaneous ventilation at 21% O_2 in our model. Thus, HBOC-201 may be a more potent tissue oxygenator and more clinically useful in HS in settings of ventilation with low FiO_2, such as in remote military operational environments.

The vasoactive properties of hemoglobin solutions result in smooth muscle contraction and consequent systemic and

Table 6 Blood Gas Data

Variable (Mean ± SEM)	Resus Group	Baseline	Post-Hemorrhage	T 30	T 45	T 60	T 90	T 120	T 150	T 180	T 240
Base excess (mmol)^	HBOC	1.2 ± 1.6	1.2 ± 1.7	−0.4 ± 0.9	0.3 ± 2.3	2.0 ± 1.7	2.1 ± 2.6	−1.1 ± 2.8	0.6 ± 1.7	2.5 ± 1.8	0.5 ± 2.3
	HEX	0.8 ± 1.1	−5.7 ± 2.0	−0.6 ± 1.9	−4.9 ± 3.3	−1.7 ± 2.6	−4.8 ± 2.8	−2.7 ± 2.1	−0.3 ± 2.4	−0.7 ± 4.5	6.5 ± 0.5
	NON	−0.6 ± 1.9	−0.5 ± 1.7	−6.8 ± 3.8	−7.3 ± 4.0	−9.3 ± 4.0	−8.0 ± 0.1	−5.4 ± 0.0	−3.9 ± 0.0	−1.6 ± 0.0	−2.6 ± 0.0
pH	HBOC	7.4 ± 0.01	7.5 ± 0.02	7.4 ± 0.01	7.4 ± 0.02	7.4 ± 0.02	7.4 ± 0.03	7.4 ± 0.05	7.4 ± 0.02	7.4 ± 0.03	7.4 ± 0.02
	HEX	7.4 ± 0.02	7.5 ± 0.03	7.4 ± 0.04	7.3 ± 0.07	7.3 ± 0.07	7.4 ± 0.51	7.4 ± 0.04	7.4 ± 0.02	7.4 ± 0.03	7.5 ± 0.02
	NON	7.4 ± 0.02	7.5 ± 0.01	7.4 ± 0.06	7.4 ± 0.09	7.3 ± 0.08	7.4 ± 0.07	7.5 ± 0.0	7.4 ± 0.0	7.4 ± 0.0	7.4 ± 0.0
Bicarbonate mmol/L^	HBOC	23.6 ± 1.3	23.3 ± 1.3	24.0 ± 0.7	26.0 ± 3.0	24.7 ± 1.6	23.5 ± 2.1	23.8 ± 2.3	25.0 ± 1.5	26.7 ± 1.6	25.1 ± 2.0
	HEX	25.1 ± 0.9	20.0 ± 1.5	24.0 ± 1.6	20.8 ± 2.4	22.9 ± 2.2	20.7 ± 2.2	22.2 ± 1.6	24.2 ± 2.0	24.2 ± 3.6	30.3 ± 0.5
	NON	24.1 ± 1.5	24.2 ± 1.4	19.7 ± 2.6	19.0 ± 2.7	17.3 ± 3.1	18.1 ± 0.1	20.0 ± 0.0	21.2 ± 0.0	23.1 ± 0.0	22.3 ± 0.0
SVO_2, %	HBOC	80.2 ± 2.3	52.9 ± 4.4#	61.6 ± 4.7	65.5 ± 6.4*#	71.9 ± 3.3*	65.8 ± 2.9	76.1 ± 4.0	73.7 ± 2.2	71.0 ± 4.0	
	HEX	78.6 ± 1.4	48.8 ± 5.4	49.4 ± 5.7	56.2 ± 9.2	36.2 ± 10.7	53.0 ± 6.1	56.2 ± 3.1	53.4 ± 0.6	65.4 ± 5.4	75.5 ± 9.1
	NON	80.3 ± 1.9	41.8 ± 5.4	32.7 ± 8.5	55.1 ± 9.9	32.2 ± 8.3	59.8 ± 0.0	52.1 ± 0.0	49.6 ± 0.0	50.7 ± 0.0	47.2 ± 0.0
PO_2, mmHg	HBOC	101.8 ± 12.8	119.2 ± 8.6	118.2 ± 9.6	113.0 ± 9.0	116.7 ± 8.9	115.8 ± 13.0	102.8 ± 8.6	101.7 ± 16.8	104.2 ± 10.8	109.9 ± 11.9
	HEX	93.7 ± 8.9	101.7 ± 10.8	100.6 ± 9.2	89.7 ± 12.3	78.9 ± 13.7	107.5 ± 7.4	109.1 ± 17.7	106.7 ± 18.8	107.2 ± 7.7	101.2 ± 11.8
	NON	97.3 ± 6.4	118.7 ± 5.8	98.9 ± 15.0	100.0 ± 11.4	80.9 ± 18.9	94.4 ± 11.7	100 ± 0.0	92.9 ± 0.0	101 ± 0.0	103 ± 0.0

* HBOC vs. HEX, $p < 0.05$.
# HBOC vs. NON, $p < 0.05$.
^ Overall $p < 0.05$.

pulmonary hypertension and GI effects, and have been linked to nitric oxide scavenging and endothelin and adrenergic receptor activation.[35–37] HBOC-201 has obvious vasoactive properties (elevated MAP, MPAP, and SVRI), but we found these to be mild and likely clinically insignificant in our model.

We were concerned that vasoactivity would result in increased bleeding and were surprised to find that in comparison with HEX and NON pigs, bleeding in HBOC-201 pigs was significantly less, when calculated per survival hour. Decreased bleeding may be related to decreased lactic acidosis in HBOC-201 pigs (i.e. improved serine protease function) and increased hemodilution in HEX pigs, but clear differences in coagulopathy laboratory parameters were observed.[38] HBOC-201 appeared to cause relative mild but detectable coagulopathy. In contrast, HEX appeared to cause relative mild thrombopathy. These observations are probably due to multiple factors (e.g. differences in hemodilution, lactic acid levels, and intrinsic properties of HEX) but do not clearly explain why blood loss was less in HBOC-201 pigs. Thus, differences in volume of fluid infused rather than coagulation change may be the main cause of the disparity in observed hemorrhage.

Renal toxicity was well documented with early generation hemoglobin solutions due to direct effects on glomeruli, as well as renal blood flow mainly due to NO binding.[39] Nephrotoxicity has not been a significant problem with second generation polymerized hemoglobin solutions (e.g. HBOC-201, Hemolink®).[40] In our model, despite lower fluid requirements in the HBOC-201 group, we found urine output indexed to survival time to be comparable with the HEX group. As urine output was almost continuous throughout the pre-hospital phase in HBOC-201 pigs, the duration of oliguria was shorter, and was manifested by improved indices of renal function.

CONCLUSIONS

Conclusions from our study should be tempered by the following limitations of our model. First, our model is specific to an abdominal solid organ injury and conclusions may not apply to other injuries (e.g. vascular, multiple organs or compartments). Second, although we choose anesthetic agents with relatively less cardiac effect (i.e. ketamine and isoflurane), adverse vasoactive effects of HBOC-201 may have been less apparent due to concomitant anesthesia. Third, interventions in this model accurately simulated pre-hospital care in the setting of delayed evacuation, but the in-hospital care capabilities were less robustly simulated. For example, the blood transfusion trigger in our model was only low Hb, which does not simulate in-hospital capability to accurately rule out hypoperfusion. Finally, as our model was designed to simulate combat care with delayed evacuation, results may not be applicable to civilian urban settings with short transport times, where HS patients would be mechanically ventilated with 100% O_2. Lastly, our study does not reflect current

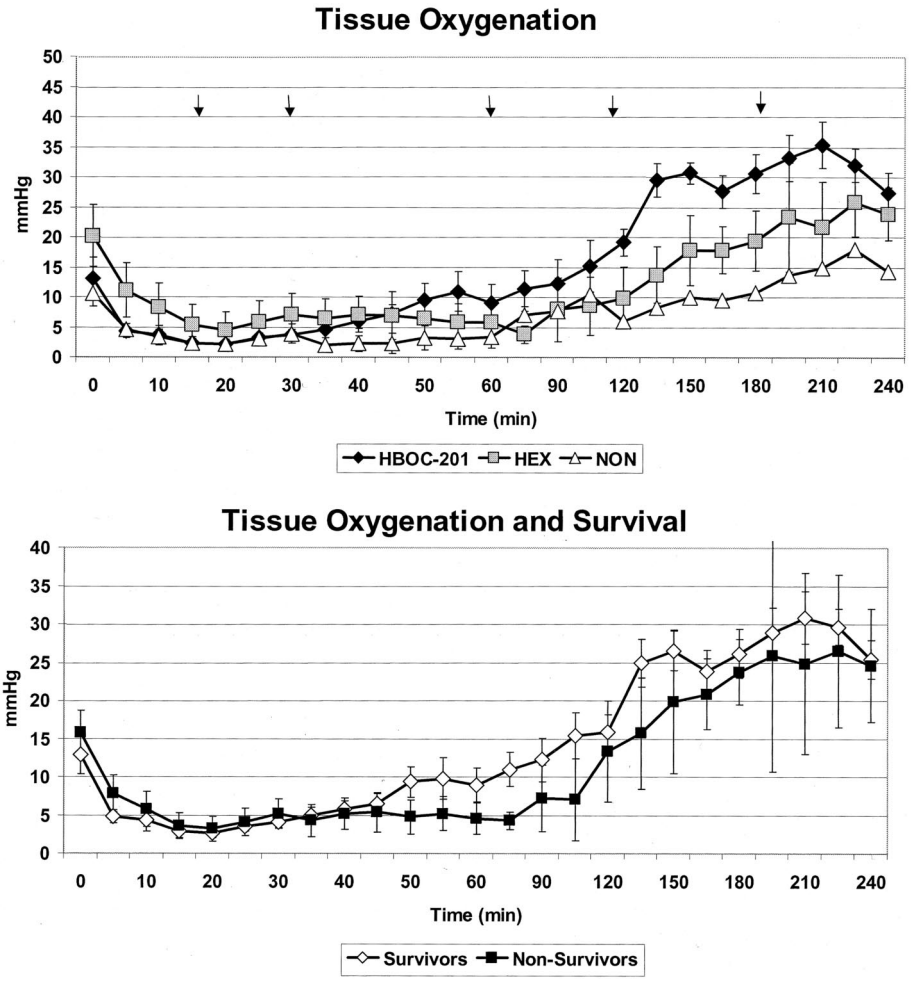

Fig. 7. *Tissue oxygenation ($tcpO_2$) as measured by transcutaneous tissue oxygen (TCOM). HBOC-201 pigs had improved tissue oxygenation compared with HEX and NON pigs* (p = *0.02) (top). Tissue oxygenation in survivors vs. non-survivors is also shown (bottom).*

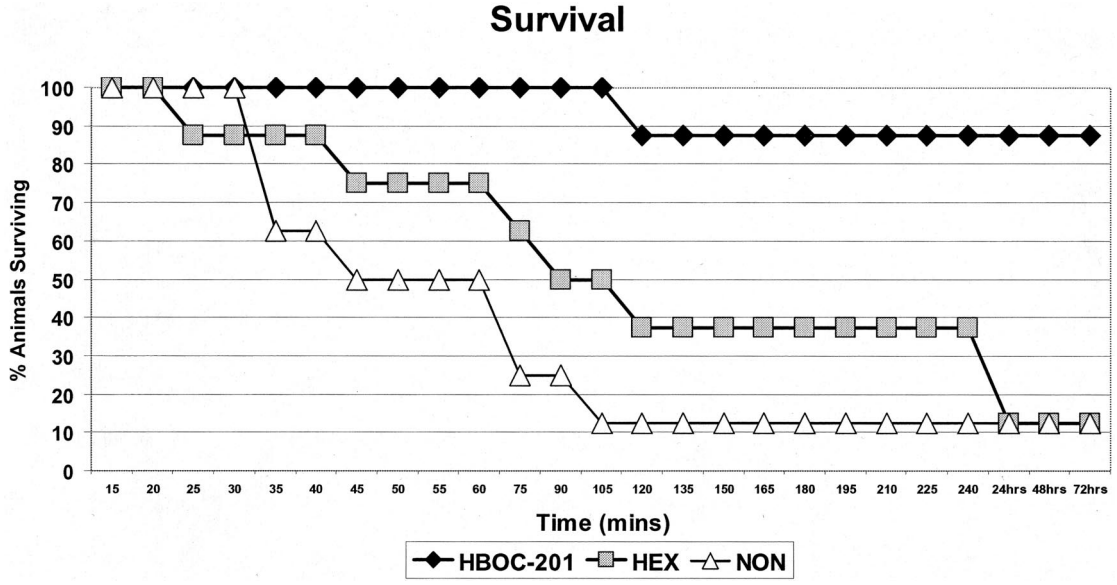

Fig. 8. *Overall survival was higher in HBOC-201–resuscitated pigs. At 4 hours (simulated hospital arrival): overall* p = *0.01, HBOC-201 vs. HEX* p = *0.12, HBOC-201 vs. NON* p = *0.01. At 72 hours: overall* p = *0.002, HBOC-201 vs. HEX* p = *0.01, HBOC-201 vs. NON* p = *0.01.*

U.S. pre-hospital care, in which crystalloid rather than colloid resuscitation is principally used.

In summary, in our swine liver injury model of uncontrolled HS with delayed evacuation, we found that hypotensive resuscitation with HBOC-201 was superior to HEX and no fluid resuscitation. Hemodynamics, tissue oxygenation, anaerobic metabolism, end-organ function, and survival were improved with HBOC-201. Despite evidence of mild vasoactivity, blood loss was not increased. Additionally, HBOC-201 was effective with lower volumes than HEX. These data are likely to be replicable in human patients with HS, and if confirmed in randomized and controlled clinical trials, suggest that hetastarch 6% may not be the optimal low-volume resuscitation fluid.

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